complement system and do not provide a specific molecular diagnosis. Most of the genetic and molecular tests are not yet available for disease characterization in clinical laboratories. Referral to research laboratories is encouraged. However, regulatory concern, access, cost, and lengthy turnaround times hamper the role that research laboratories will play in clinical practice.

With the defects of aHUS being characterized in an increasing number of cases at the genetic and molecular levels, clinicians face a mounting challenge of deciding how to apply the vast albeit incomplete knowledge to the management of individual patients. In this regard, measures to block complement activation with inhibitors such as eculizumab seem attractive for the treatment of aHUS.[12,13] Nevertheless, clinical experience with this therapeutic modality is quite limited for aHUS, and serious questions remain unanswered. It is hoped that with further elucidation of the molecular mechanisms, better measures will become available in the near future to tackle the serious business of aHUS.

DISCLOSURE
The author declared no competing interests.

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REFERENCES

1. Tsai HM. Mechanisms of microvascular thrombosis in thrombotic thrombocytopenic purpura. *Kidney Int Suppl* 2009; **75** (Suppl 112): S11–S14.
2. Skerka C, Jozsi M, Zipfel PF *et al.* Autoantibodies in haemolytic uraemic syndrome (HUS). *Thromb Haemost* 2009; **101**: 227–232.
3. Delvaeye M, Noris M, De VA *et al.* Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **361**: 345–357.
4. Fremeaux-Bacchi V, Dragon-Durey MA, Blouin J *et al.* Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. *J Med Genet [online* 2004; **41**: e84; doi:10.1136/jmg.2004.019083.
5. Abramson N, Alper CA, Lachmann PJ *et al.* Deficiency of C3 inactivator in man. *J Immunol* 1971; **107**: 19–27.
6. Bienaime F, Dragon-Durey M-A, Regnier CH *et al.* Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. *Kidney Int* 2010; **77**: 339–349.
7. Dragon-Durey MA, Blanc C, Marliot F *et al.* The high frequency of complement factor H related CFHR1 gene deletion is restricted to specific subgroups of patients with atypical haemolytic uraemic syndrome. *J Med Genet* 2009; **46**: 447–450.
8. Heinen S, Hartmann A, Lauer N *et al.* Factor H-related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation. *Blood* 2009; **114**: 2439–2447.
9. Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. *Nat Rev Immunol* 2009; **9**: 729–740.
10. Rose KL, Paixao-Cavalcante D, Fish J *et al.* Factor I is required for the development of membranoproliferative glomerulonephritis in factor H-deficient mice. *J Clin Invest* 2008; **118**: 608–618.
11. Spencer KL, Hauser MA, Olson LM *et al.* Deletion of CFHR3 and CFHR1 genes in age-related macular degeneration. *Hum Mol Genet* 2008; **17**: 971–977.
12. Nurnberger J, Philipp T, Witzke O *et al.* Eculizumab for atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **360**: 542–544.
13. Gruppo RA, Rother RP. Eculizumab for congenital atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **360**: 544–546.

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Functional evidence confirmed by histological localization: overlapping expression of erythropoietin and HIF-2α in interstitial fibroblasts of the renal cortex

Hayo Castrop[1] and Armin Kurtz[1]

Erythropoietin (EPO) is the central hormone in the regulation of erythropoiesis. Synthesis of EPO and its constitutive release into the circulation are controlled by the transcriptional activity of the *EPO* gene. A key regulator of *EPO* is the dimeric hypoxia-inducible transcription factor (HIF), which has three isoforms. Paliege and co-workers provide convincing morphological evidence that it is the HIF-2 isoform that essentially triggers *EPO* in the kidney, which is the primary source of EPO in the body.

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During hypoxic challenges, the reduced availability of oxygen triggers an increase in erythropoiesis of the organism, which results in an augmented oxygen-carrying capacity. In mammalians, this response to hypoxic stimuli is mediated by the erythropoiesis-stimulating hormone erythropoietin (EPO). With the exception of a few hepatic EPO-producing cells, the bulk of systemic EPO in adult mammals is generated in the renal cortex.[1,2]

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Increased production of EPO in response to hypoxic stimuli is virtually entirely due to enhanced transcriptional activity of the *EPO* gene. The functional linkage between changes in tissue oxygen tension and altered EPO transcription is provided by the hypoxia-inducible transcription factors (HIFs).

HIF is a heterodimeric protein consisting of an α- and a β-subunit. Three different α-subunits of HIF have been described (HIF-1α, HIF-2α, and HIF-3α). The various HIFα subunits are regulated by oxygen tension at the protein level, whereas the β-subunit is constitutively expressed. In normoxia, HIFα is continuously synthesized; however, steady-state levels in the cells are low, as HIFα is rapidly

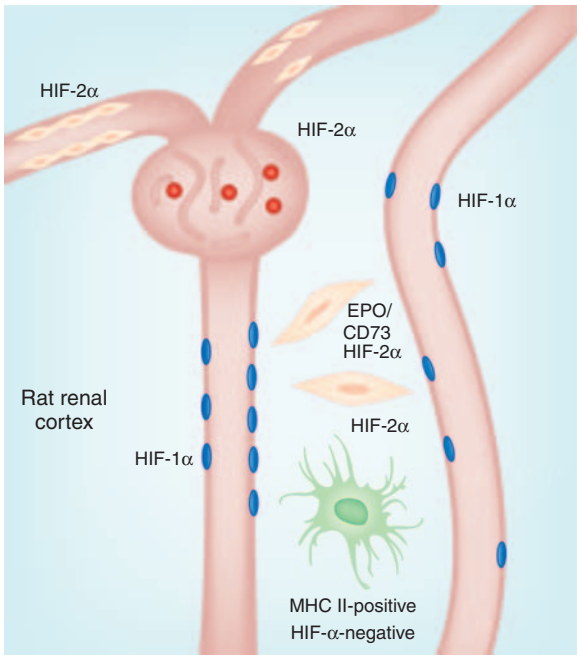

Figure 1 | HIF-α expression in the rat renal cortex under hypoxic conditions.[12] HIF-1α expression was restricted to the tubular system. HIF-2α was found in interstitial fibroblasts and in endothelial and glomerular cells. HIF-2α expression overlapped with EPO expression in ecto-5′-nucleotidase/CD73-positive interstitial fibroblasts. Interstitial MHC-II-positive cells (dendritic cells, macrophages) showed no HIF-2α expression. EPO, erythropoietin; HIF, hypoxia-inducible transcription factor.

ubiquitinated and targeted to proteasomal degradation. This degradation of HIFα is mediated by the interaction of HIFα with the tumor suppressor von Hippel–Lindau protein (pVHL), which specifically interacts with hydroxylated HIFα. Hydroxylation of HIFα, in turn, depends on the presence of oxygen as a substrate for HIF prolyl-hydroxylases. In hypoxia, the interaction between HIFα and pVHL is suppressed because of reduced HIF hydroxylation; consequently, HIFα accumulates in the cell, interacts with the constitutively expressed β-subunit, translocates to the cell nucleus, and eventually binds to hypoxia-responsive elements in target gene promoters.[3] *EPO* is by far not the only HIF-dependent gene; in fact, more than 150 genes are known to possess functionally relevant hypoxia-responsive elements in their promoters.[4]

So, what is the reason for the evolution of different types of HIFα subunits? In view of the large array of HIF target genes, it appears reasonable to assume that different HIFα subunits may allow the regulation of oxygen-dependent genes in a locally defined manner. Thus, HIFα expression follows distinct cell- and tissue-specific patterns; HIF-1α is rather ubiquitously expressed, whereas HIF-2α expression is more restricted to the endothelium of blood vessels and distinct cells of the kidney, brain, heart, lung, liver, pancreas, and intestine.[5] Compared with HIF-1α and HIF-2α, relatively few data are available regarding the biological function and exact localization of HIF-3α.

Both HIF-1α and HIF-2α are expressed in the kidney and, in principle, may account for hypoxia-induced EPO expression. Although there are data to support a role of both HIFα subunits in the regulation of EPO expression *in vitro*, there is now compelling evidence that—at least in the adult organism—HIF-2α is the crucial factor driving EPO transcription *in vivo*.[6,7] Since classical knockout models of either HIF-1α or HIF-2α are not viable,[8] an elegant mouse model with inducible inactivation of HIFα was developed. In this mouse strain, acute HIF-2α deletion was obtained in adult mice by inducible gene targeting using the tamoxifen-Cre system.[7] Acute loss of HIF-2α resulted in anemia in tamoxifen-treated transgenic mice. Furthermore, activation of EPO transcription during hypoxia was substantially reduced in HIF-2α conditional knockout mice, whereas conditional deletion of HIF-1α was essentially without effect on the induction of EPO under hypoxia.[7] These data were further confirmed by evaluation of HIF-2α-deficient mice, which turned out to survive at a low percentage when backcrossed into a specific genetic background.[9] HIF-2α$^{-/-}$ mice were anemic and showed markedly reduced renal EPO expression.[9] In summary, the mentioned studies, accompanied by convincing *in vitro* evidence, strongly indicate that HIF-2α rather than HIF-1α is relevant for the control of renal EPO expression.

A functional relevance of HIF-2α for the direct control of renal EPO expression of course requires that the transcription factor component HIF-2α is actually present in EPO-expressing cells. Renal EPO expression has been shown for a subset of renal cortical interstitial cells, which coexpress ecto-5′-nucleotidase.[10] Similar, HIF-2α was shown to be expressed in renal cortical interstitial cells, although the exact nature of these cells and the question of a possible coexpression of HIF-2α in EPO-producing cells remained unsettled. Conversely to HIF-2α, HIF-1α was detected predominantly in the tubular system.[11] Thus, the remaining piece of the puzzle still was to be added: the unambiguous demonstration of a coexpression of HIF-2α and EPO mRNA in the same renal cortical interstitial cell type.

The study by Paliege *et al.*[12] (this issue) now offers new anatomical insights regarding the distribution of HIF-1α, HIF-2α, and EPO in the rat kidney. It essentially confirms the functional data, which had suggested a key role for HIF-2α (and not HIF-1α) in the control of EPO expression in the kidney. In line with being highly hypoxia-inducible proteins, HIF-1α, HIF-2α, and EPO were virtually undetectable under normoxic conditions. Then, two experimental maneuvers were used to mimic hypoxic conditions: rats were either kept under isobaric hypoxia (8% O_2 for 6 hours) or treated with the prolyl-hydroxylase inhibitor FG-4497. For the two maneuvers, the results were essentially identical: EPO expression was induced under 'hypoxic' conditions in interstitial cells of the renal cortex with a higher density of EPO-positive cells in deeper than in more superficial portions of the cortex. Whether this heterogeneous

distribution within the renal cortex reflects some gradient of oxygen tension was not addressed by Paliege *et al.*[12] but it may appear unlikely, since protein levels of HIF-2α were similar in all portions of the renal cortex. Thus, HIF-2α was found predominantly in interstitial cells of the renal cortex. In addition, HIF-2α was detected in some scattered endothelial and glomerular cells. As the key finding, there was a marked overlap of EPO- and HIF-2α-positive cells: between 83 and 91% of EPO-producing interstitial cells also stained positive for HIF-2α; the degree of coexpression was slightly higher in the deeper than in the more superficial cortex. Thus, although the vast majority of EPO-positive cells also expressed HIF-2α, there was apparently a subset of EPO-expressing cells that did not stain for HIF-2α. Whether this finding was based on a sensitivity issue of HIF-2α detection or may indicate that EPO-producing cells consist of two sub-populations—the larger one being HIF-2α-dependent and the smaller one being HIF-2α-independent—was not addressed by Paliege *et al.*[12]

Conversely to HIF-2α, HIF-1α was detected in epithelial cells, and there was no colocalization with EPO-expressing interstitial cells. Thus, the findings of this localization study confirm previous functional evidence indicating that HIF-2α is the major factor in oxygen-dependent EPO expression. Although the absence of HIF-1α from EPO-expressing cells of the renal cortex excludes a functional relevance of HIF-1α for a direct control of EPO transcription, HIF-1α is well able to drive EPO expression in extrarenal tissues.[13,14]

Paliege *et al.*[12] furthermore attempted to characterize the nature of HIF-2α- and EPO-coexpressing interstitial cells. As a marker for interstitial fibroblasts of the renal cortex, they used ecto-5′-nucleotidase.[10] Most HIF-2α- and EPO-positive cells also stained for ecto-5′-nucleotidase, whereas no coexpression with the dendritic-cell marker major histocompatibility complex II was detected, suggesting—but not proving—that the oxygen-sensitive interstitial cells of the renal cortex mainly represent fibroblasts (Figure 1).

In summary, the convincing localization study by Paliege *et al.*[12] now adds the last piece to the puzzle and provides a convincing—anatomical—basis for what we had expected from functional studies: HIF-2α is an indispensible prerequisite for oxygen-dependent EPO expression in the adult kidney.

DISCLOSURE
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REFERENCES

1. Maxwell PH, Osmond MK, Pugh CW *et al.* Identification of the renal erythropoietin-producing cells using transgenic mice. *Kidney Int* 1993; **44**: 1149–1162.
2. Maxwell PH, Ferguson DJ, Osmond MK *et al.* Expression of a homologously recombined erythopoietin-SV40 T antigen fusion gene in mouse liver: evidence for erythropoietin production by Ito cells. *Blood* 1994; **84**: 1823–1830.
3. Ivan M, Kondo K, Yang H *et al.* HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* 2001; **292**: 464–468.
4. Semenza GL. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. *Annu Rev Cell Dev Biol* 1999; **15**: 551–578.
5. Wiesener MS, Jurgensen JS, Rosenberger C *et al.* Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *FASEB J* 2003; **17**: 271–273.
6. Scortegagna M, Morris MA, Oktay Y *et al.* The HIF family member EPAS1/HIF-2alpha is required for normal hematopoiesis in mice. *Blood* 2003; **102**: 1634–1640.
7. Gruber M, Hu CJ, Johnson RS *et al.* Acute postnatal ablation of Hif-2alpha results in anemia. *Proc Natl Acad Sci USA* 2007; **104**: 2301–2306.
8. Peng J, Zhang L, Drysdale L, Fong GH. The transcription factor EPAS-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. *Proc Natl Acad Sci USA* 2000; **97**: 8386–8391.
9. Scortegagna M, Ding K, Zhang Q *et al.* HIF-2alpha regulates murine hematopoietic development in an erythropoietin-dependent manner. *Blood* 2005; **105**: 3133–3140.
10. Bachmann S, Le Hir M, Eckardt KU. Co-localization of erythropoietin mRNA and ecto-5′-nucleotidase immunoreactivity in peritubular cells of rat renal cortex indicates that fibroblasts produce erythropoietin. *J Histochem Cytochem* 1993; **41**: 335–341.
11. Rosenberger C, Mandriota S, Jurgensen JS *et al.* Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. *J Am Soc Nephrol* 2002; **13**: 1721–1732.
12. Paliege A, Rosenberger C, Bondke A *et al.* Hypoxia-inducible factor-2α-expressing interstitial fibroblasts are the only renal cells that express erythropoietin under hypoxia-inducible factor stabilization. *Kidney Int* 2010; **77**: 312–318.
13. Stolze I, Berchner-Pfannschmidt U, Freitag P *et al.* Hypoxia-inducible erythropoietin gene expression in human neuroblastoma cells. *Blood* 2002; **100**: 2623–2628.
14. Grimm C, Wenzel A, Groszer M *et al.* HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. *Nat Med* 2002; **8**: 718–724.

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KDIGO guideline for the care of kidney transplant recipients will be resource challenged

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The Executive Summary of the KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients is presented in this issue of *Kidney International*. This guideline will require careful attention for implementation in the context of available resources within each country, especially those with underdeveloped economies.

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In this issue of *Kidney International*,[1] the Executive Summary of the KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients is presented to the international community. The complete report, including the rationale and references for each recommendation, is to be published in the *American Journal of Transplantation* (*AJT*).[2] The complete report is also available on the websites of KDIGO (Kidney Disease: Improving Global Outcomes)

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